

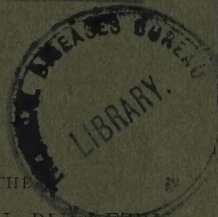
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The Etiology of Yellow Fever

BY

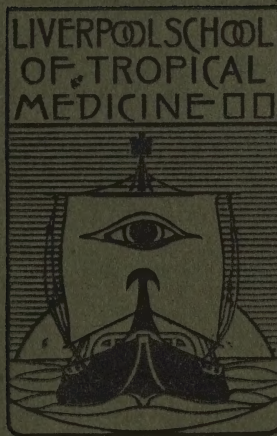
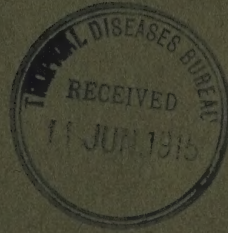
HARALD SEIDELIN

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THE ETIOLOGY OF YELLOW FEVER

BY HARALD SEIDELIN.

Earlier authors admit different modes of transmission of yellow fever and discuss especially the question, whether the disease is contagious or not. Some observers, for instance, Arejula (1806) and Audouard (1822), consider the air as an important, or the important means of transmission, though, of course, without any clear conceptions as to how the transport of the infection through the atmosphere would take place.

In the modern period of microbiology it has been accepted almost universally that a specific micro-organism must be supposed to be the cause of yellow fever. This taken for granted, the problem could be formulated more precisely; the theories of transmission had now to explain how the pathogenic microbe could gain access to the body.

Advocates of the bacterial origin of yellow fever, especially Sanarelli (1897), are inclined to consider the digestive tract as the primary place of infection. This theory is apparently supported by the finding of typical lesions in this tract, and is also defended on epidemiological grounds, for instance by Strain (1899). Other authors, as Azevedo Sodré and Couto (1901), believe that the penetration of the virus into the body takes place through the respiratory organs, thus combining in the apparently most logical way the theory of aerial transmission with that of bacterial origin.

The conveyance of the infection through the air could, however, also be explained if insects served as carriers and the possibility of such a means of transport had presented itself, already at a much earlier time, to the minds of various observers in different countries, in a more or less indefinite way.

The first, however, who formulated a definite theory to this effect was, as far as we know, Beauperthuy (quoted by Boyce, 1909). His manner of reasoning testifies to a great power of observation, but his hypothesis was soon forgotten,

and seems to have remained unknown to the next great promoter of the mosquito-theory, Finlay.

Finlay published from 1883 to 1899 several important papers on this subject, definitely setting forward his theory and expressing a firm conviction of yellow fever being transmitted by means of mosquitos. He indicated the species of mosquito which he believed to serve as transmitting agent, and undertook experiments with the object of proving his theory. The mosquito with which Finlay experimented was known at that time by the name of *Culex mosquito*, a synonym of *Stegomyia fasciata*, Fabr., and Finlay's description makes it quite clear that he really worked with the *Stegomyia*. He claimed to have produced in a number of cases yellow fever by mosquito-inoculation, but the results of later investigations oblige us to entertain serious doubt as to the correct interpretation of his experiments. Finlay's name and work should, however, always be remembered, so much the more since the mosquito experiments of the American Military Commission were undertaken at his instigation. According to Guiteras (1910), the mosquitos used in these experiments were actually supplied by Finlay. Due honour has been accorded to Finlay by most modern authors, but, unfortunately, not by all; his name has been mentioned on several occasions both by Reed (1901) and by Carroll (1905), but, most unjustly, in the well-known resumé of the work of the Commission, by Reed (1902), it was entirely omitted. Finlay even went so far as to draw from his standpoint the logical deductions with regard to prophylaxis, and recommended in the clearest possible terms the establishment of anti-mosquito measures as effective means of combating yellow fever (1899).

The work of the United States Army Commission, consisting of Reed, Agramonte, Carroll, and Lazear, is too well known to require any detailed reference. The results obtained have been embodied in various papers by Reed and by Carroll (1901-1902). From this time the transmission of yellow fever by means of mosquitos has come to be considered an established fact.

Further contributions to the question of transmission and to the biology of the transmitting agent have been made by Guiteras (1901), Durham and Myers, published by Durham (1902), Marchoux, Salimbeni and Simond (1903), Marchoux and Simond (1906), Otto and Neumann (1905), and others.

Instead of proceeding to a historical review of this well-known subject, it may be of interest to recapitulate what parts of the now established theory may be considered finally proved, and what parts are still open to discussion. It will be necessary also to consider the proofs which have led to the acceptance of certain doctrines. This summary will help to point out the lines on which further researches ought to proceed.

I. The fundamental doctrine that yellow fever can be transmitted through the bite of *Stegomyia fasciata* rests mainly upon the experiments conducted by Reed, Carroll and Agramonte. According to Carroll (1903) positive results were obtained in fourteen cases out of a total of twenty-three inoculations which were believed to fulfil the conditions necessary for successful experiments. As regards these results objection has been raised because the symptoms produced in the individuals submitted to inoculations were not characteristic of yellow fever and, especially, because all the cases were remarkably light. This objection does not appear to be valid; it is true that a series of fourteen cases of yellow fever without a death is an exceptional occurrence, but at least some of the cases presented evidently quite typical symptoms. It must be remembered also, that a comparatively favourable prognosis might reasonably be expected in experimental cases, because the patients would be submitted from the beginning of the disease to the best possible treatment, and probably even be somewhat cautious in their manner of living during the stage of incubation. Now, taking it for granted that the ailment produced was really yellow fever, the whole value of the experiments depends on the question whether it can safely be asserted that the infection could have been contracted in no other way than

through the experimental inoculation. We know that the individuals experimented upon were isolated, during the experiments, and some time before, in Camp Lazear, at some distance from Habana. There was evidently no possibility of contact-infection, but, on the other hand, many earlier writers have denied or laid very little stress upon contact-infection in yellow fever. Nor is the importance very great of the negative experiments made at the same time with 'fomites'—bed-clothes, etc., used by yellow fever patients, and partly soiled with their blood and discharge. The real point at issue is whether there existed any possibility of infection through water or air, the two media which were at that particular time considered most likely to convey the infection. As no information is given about any special precautions against such infection, except the somewhat isolated situation of the camp, the inference is probably justifiable that none were taken. The experiments were not made under ideal conditions, but the possibility of erroneous interpretation of the results is greatly diminished by the fact that several non-immune individuals inhabiting the camp at the same time, without being experimented upon, remained healthy. However, the experiments can never be considered absolutely conclusive, and the transmission of yellow fever by means of *Stegomyia fasciata* was not proved beyond doubt, as the transmission of malaria by Anophelines, and of papataci fever by *Phlebotomus* has been proved. Both in the case of malaria and in that of papataci fever positive results were obtained in transmission experiments conducted where no other possibility of infection existed, namely, in the first case in Berlin-Londón (Manson), and in the second in Vienna (Doerr), and also in London (Birt). Moreover, in malaria the development of the parasites during their life in mosquitos was followed, by Ross and others. In yellow fever both these kinds of proof might apparently be obtainable, but in practice it would be difficult to secure an individual living outside the infected areas, and nevertheless willing to submit to an inoculation. It would, perhaps, not even be justifiable

to attempt this experiment, though, on the other hand, it might be argued that it would be advantageous for a would-be emigrant to a yellow fever place to acquire immunity beforehand; he would not unlikely be under better conditions at home, and the risk would consequently be less than that of an ordinary attack of the disease. The second means of securing absolute proof remains, at any rate, available, and the attempt to obtain it should be made with all energy in spite of the great difficulties which present themselves. The parasite of yellow fever is certainly very small when living in human blood; it may be that it is smaller still when in the mosquito.

No absolute proof exists, then, of the mosquito transmission of yellow fever, but the evidence brought forward is very strong, especially as positive results have also been obtained by Guiteras (1901), Marchoux, Salimbeni and Simond (1903), and others. According to Carroll (1905), fifteen positive cases have been added later to the fourteen obtained by the American Commission. Finlay's positive experiments are not included here, because their significance is now generally contested. It must, however, be remembered that the same objections which are raised, for instance by Carroll (1905), against the experiments of Finlay, have been made by others, as Sanarelli (1904), to the experiments of the American Commission, especially that the disease might have been contracted otherwise than through the experimental inoculations.

The most important corroboration which the doctrine has received is, perhaps, that furnished by the results which were obtained in Habana and Rio de Janeiro, when anti-mosquito measures were put in force. Earlier attempts to combat yellow fever, according to the experience gained in campaigns against other infectious diseases, had proved futile; but hand in hand with the extinction of mosquitos came a rapid decrease in the number of yellow fever cases, and the disease soon disappeared from both the cities named. In Habana there has since then only been a single outbreak of short duration, and in Rio the disease seems to have lost all

importance, although an occasional case has occurred now and then. The results of the anti-mosquito campaigns in the Isthmian Canal Zone and in New Orleans constitute proofs of a similar nature.

We are compelled to conclude that the transmission of yellow fever by means of mosquitos has never been proved beyond discussion, but that there are exceedingly strong arguments in favour of the theory. It is probable that the absolute proof could be given, and it is most urgent to attempt to give it in order to silence all dispute about this point.

II. When the foundation of a theory is not absolutely firm it is clear that extreme care should be exercised in the working out of details. The first investigators took great care not to express themselves in too definite terms, but later authors have, unfortunately, not always done the same. It is curious—and deplorable—to see how mere suppositions have, through frequent repetition, been transformed into indisputable dogmas. Thus, Reed says (1902), 'whether other species of mosquitos than *Stegomyia* are capable of conveying the parasite of yellow fever has not, as yet, been determined by the Commission.' The only important contributions to this question which have been made since then, are those of Marchoux, Salimbeni and Simond (1903), and Marchoux and Simond (1906), who experimented with several other species of mosquitos and, failing to obtain positive results, concluded, 'Il est donc très probable que le virus amaril est adapté à l'organisme chez cette unique espèce (*Stegomyia*) à l'exclusion de tous les autres culicides.' Negative experiments prove very little, unless overwhelmingly numerous, and those of Marchoux and Simond are not numerous at all; moreover, the number of species with which they experimented was small, being restricted to the mosquitos which were common in Rio de Janeiro. In fact, their researches represent a practical study of the mosquitos responsible for the prevalence of yellow fever in Rio, rather than a thorough investigation of the question. One of the reasons, for which Marchoux and Simond consider it probable

that *S. fasciata* is the only mosquito capable of transmitting yellow fever, is that the females of all other species experimented with died soon after having laid eggs. This has been said about many mosquitos, and afterwards it has been found not to be the case, but even accepting it for the species observed, the same reasoning would evidently not apply to malaria-transmitting mosquitos, and is consequently of very little value as an argument in favour of excluding all other mosquitos. Marchoux and Simond do not go farther than to state a probability, but in more recent literature it is often laid down as an indisputable fact that *Stegomyia fasciata* alone, of all mosquitos, transmits yellow fever. This statement may be correct, but it is entirely unproved, and it should be remembered, in particular, that nothing is known about negative experiments with other species of *Stegomyia*. The question is evidently one of great epidemiological interest.

III. Finlay (1886) claimed to have obtained positive results by inoculation as early as two days after the mosquito employed had bitten a yellow fever patient. Reed and his collaborators failed to obtain positive results when only ten days or less had elapsed from the time when the mosquito had bitten a yellow fever patient until it was allowed to bite a healthy individual. Here, again, the positive results would prove more than the negative ones, were it not for the fact, admitted by Finlay (1901), that his experiments were conducted under conditions which made it an impossibility to safeguard entirely against accidental infections. Marchoux, Salimbeni and Simond (1903), are generally quoted as authorities for this part of the doctrine, and it is true that they have made a statement to that effect, but they seem to have drawn the conclusion from the facts published by the American Commission; at least, I do not find in their own report any experiments which bear directly upon this question. Even in their later papers, Reed (1902) and Carroll (1905) do not draw any definite conclusion from their own observations, and in this evidently take the only correct position, since it is admitted by all investigators that

experiments may fail under conditions likely to assure positive results. In another place, Carroll (1903) says that he believes it to be 'impossible to confer protection or to produce infection of any grade, with an insect that has been contaminated less than a week.'

It is obvious that it is, at present, impossible to lay down any definite time-limit; nevertheless, it is now everywhere in yellow fever literature taken for granted that the mosquito can only transmit yellow fever after a period of at least twelve days since it sucked infected blood; this is taken for granted, simply because it has once been said, though without any proof, and afterwards repeated again and again.

The inference has been made next, and quite naturally, that the yellow fever parasite undergoes an evolution of twelve days' duration in the *Stegomyia fasciata*. This remains to be investigated.

IV. Reed (1902) concludes, from experiments with direct injection of blood, that 'the specific agent of yellow fever is present in the blood, at least during the first, second and third days of the attack.' He had also failed to obtain positive results by mosquito inoculation, when the mosquito had sucked blood from a yellow fever patient at a later stage than on the third day.

The definite statement has, in this case also, been made by Marchoux, Salimbeni and Simond (1903), on the strength of the following experiments (p. 677):—

1. Injection of 6 c.c. of serum taken from a patient with severe yellow fever, on the 8th day, diluted with five times its volume saline solution, and passed through a Berkefeld filter.
 2. Injection of $4\frac{1}{2}$ c.c. serum from a case on the 4th day, filtered through Chamberland filter F, but not diluted.
 3. Injection of 1 c.c. serum (4th day), diluted with four volumes saline solution, and passed through a Berkefeld filter.
 4. Injection of $\frac{1}{2}$ c.c. undiluted and unfiltered serum (4th day).
- The result was negative in all cases.

The authors conclude briefly: 'Il n'y a donc plus de microbes dans le sang à partir du 4^e jour de la maladie.' This conclusion seems to me entirely unwarranted, and,

because of the importance of the question, I shall point out several possibilities of error.

1. The serum has, in three out of the four cases, been filtered before injection. Now, the same investigators have obtained positive results with filtered serum, but only in the case of bacterial infection can it be asserted that an organism which is sometimes capable of passing through a filter should always be able to do so. A protozoon would easily differ so much in size in its different stages of evolution that it might, in some cases, pass through a filter, but in others not; moreover, it might in some stages be extra-, but in others intra-corpuseular, and would in the latter stages, of course, be retained by the filter.

2. In the fourth case, the only one in which unfiltered serum was used, the quantity injected was very small, only 0.5 c.c. The objection that this amount might be insufficient is not purely theoretical, since we know from certain protozoal diseases that considerable quantities of blood may be necessary to produce an infection; Schilling, for instance, states (1908) that he obtained positive results by the injection of 10 to 20 c.c. of blood in nagana-experiments, although the injection of 2 c.c. had proved negative.

3. Injections of blood instead of serum were not made; thus no positive results could be obtained in the case that all parasites on the fourth day were intra-corpuseular, or there might even be present extra-corpuseular, but exclusively sexual forms.

4. The authors proceed: 'Une disparition si brusque de microbes si nombreux ne doit pas se produire sans qu'il reste dans le sérum des anticorps actifs.' And they believe that they have demonstrated the presence of such anti-bodies. It is not stated how the knowledge had been acquired that the microbes were very numerous at some earlier period, but the most remarkable fact is that the question of such anti-bodies is not further considered. On pages 672-676 the possible presence of anti-bodies, together with microbes during the earlier days of the disease is discussed, and on pages 677-679

that of anti-bodies after the disappearance of the virus and during convalescence. It seems, therefore, only too probable that there might be a transitional stage, during which microbes would still exist in the blood, but at the same time anti-bodies in sufficient quantity to impede their development, when assisted by the natural resistant power of a fresh human organism. This objection is mentioned because it presents itself as a natural deduction from the authors' statements, but, as a matter of fact, I regard the other objections just made as being of a much more serious nature.

5. Only one of the individuals experimented upon (experiment with *filtered* serum) was afterwards shown to be susceptible to the infection. In other experiments the authors lay stress upon eliminating the possibility of a pre-existing immunity; it is, therefore, somewhat unfortunate that it was not possible to do so in the remaining three cases (or perhaps only two; in one of the experiments it is not stated whether the individual was tested or not).

6. Finally, blood injection experiments prove nothing with regard to mosquito transmission. In direct transmission the parasites responsible for the infection would probably be indifferent forms, but those infecting mosquitos, sexual. The absence of the former would not prove that the latter had been absent as well.

At the end of the chapter in question, the authors say:—

'Nous sommes donc autorisés à regarder comme valables les conclusions que nous avons tirées de nos expériences.

'On nous pardonnera de les avoir généralisées malgré le petit nombre de celles-ci, étant donné qu'elles ont été faites sur l'homme.'

This reason adequately explains why more numerous experiments have not been made, but it can never justify the establishment of otherwise unwarranted conclusions.

It can, consequently, not be considered proved that the blood of a yellow fever patient is infective during the first three days only; much less can it be concluded that the parasite must be absent from the blood after that time.

V. The transmission of the yellow fever virus from an infected mosquito to its offspring has been observed by Marchoux and Simond (1906, pp. 20-22) in one experiment, and is by Otto (1907) regarded as proved. In the experiment referred to, the resulting attack of fever was very slight, but probably the diagnosis was a correct one. A single experiment is, as a rule, not considered to possess the importance of a decisive proof, but in this case the whole value of the experiment depends on the question of isolation; if all other possibilities of infection had been excluded, the case is, to say the least, very significant. Unfortunately the statements with regard to this particular are not quite clear, but the conditions of isolation have certainly not been ideal, since the possibility of casual infection is discussed (on p. 22), though only to reject it. The patient lived at the laboratory, but evidently at the laboratory in Rio (p. 22 and 192), not in the yellow fever free Petropolis, where the earlier experiments of the Commission took place. The French observers, themselves, seem inclined to regard hereditary transmission as an exceptional occurrence, but are certainly right in considering it also an important point for further epidemiological studies.

VI. The same French authors describe infection by means of laboratory-bred mosquitos fed on sugar-water in which infected mosquitos had been triturated. A positive result was obtained in one case only (p. 34), and the question whether the individual was efficiently isolated again arises. In view of the experiment on hereditary transmission just referred to, it might also be questioned whether it could be excluded that some of the mosquitos employed in the experiment might be the offspring of infected mosquitos.

Marchoux and Simond are of opinion that infection from dead mosquitos to other mosquitos is possible in laboratory experiments only, and does not take place in nature.

VII. Through a series of interesting and well-conducted experiments Marchoux and Simond (1906) have arrived at the conclusion that young females of *Stegomyia fasciata*, when they suck blood for the first time, bite indiscriminately

in the day time and during the night; later on, and especially after having laid eggs, they bite only during the time from late afternoon until early in the morning. The authors explain thus the fact, to which attention had been drawn by Azevedo Sodré and Couto (1901) and others, that yellow fever seems to be contracted always at night time, and especially that inhabitants of the elevated and yellow fever free Petropolis, near Rio de Janeiro, though going down to the city for the day, do not become infected if they only take care to return always before sunset.

On the other hand, it cannot be left out of consideration, that Finlay (1886) and Durham (1902) expressly state that the *Stegomyia fasciata* is a day-mosquito. My own experience, like that of most observers, is that it bites both at day and at night-time, but that it is particularly troublesome in the afternoon about 3 to 6; as to the time when the older females bite, I have made no observations.

The whole discussion may be thus summarized: The transmission of yellow fever by means of *Stegomyia fasciata* has not been proved according to strict scientific principles, but has been made probable to such a degree that it can be taken for granted for all practical purposes. All prophylactic measures, in particular, may safely take the form of anti-mosquito measures, as this form of campaign has proved efficient in practice.

All the other parts of the doctrine need further confirmation before they should be finally accepted. A full investigation of all, except point VII, will be possible when the evolution of the parasite can be followed. Point VII must be investigated through further biological observations on mosquitos. The study of all the points could be advanced materially through animal experiments, which were inaugurated by Thomas (1909), but have so far not been made use of to solve any of the debatable questions.

The actual knowledge of insect-borne diseases makes it almost certain that the acceptance of the mosquito theory excludes—experimental work excepted—the possibility of other means of transmission.

II. THE PARASITE

During the early microbiological era yellow fever, like most other infectious diseases, was believed to be of bacterial origin. Numerous bacilli and cocci have been described in the belief that they represented the specific yellow fever germ. Reviews of the corresponding literature are found in the treatises of Bérenger-Féraud (1890) and Azevedo, Sodré and Couto (1901). Most of these organisms have now fallen completely into oblivion, though great claims were made for some of them, and a considerable amount of material was brought forward, especially by Freire (1885, 1898) in support of his *Cryptococcus xanthogenes*, and by Sanarelli (1897) to prove the etiological rôle of the microbe described by him under the name of *Bacillus icteroides*.

A short mention of the latter bacillus may be of interest. It was found by Sanarelli in seven out of twelve cases, and its morphological and biological characters were carefully studied. His results were confirmed by many observers both in Brazil, Mexico, Cuba and the United States; a review of the corresponding literature is to be found in the treatise on yellow fever by Azevedo Sodré and Couto (1901). The discovery, on the other hand, soon met with considerable opposition, especially from Sternberg (1899) and his collaborators. Then came the work on mosquito transmission, already discussed, and after that time one observer only Bandi (1904), has taken up the defence of *Bacillus icteroides* as an etiological factor.

Bandi's arguments cannot be taken as decisive, and his observations do not prove that *Bacillus icteroides* is the cause of yellow fever. They confirm, however, the fact that it is frequently found during the second period of the disease in the blood, and on post-mortem, both in the blood and in various organs. Considering this, and the characteristic phenomena observed in experimental inoculations by Sanarelli, Azevedo Sodré and Couto, Bandi and others, a certain amount of evidence cannot be denied to exist, that probably *Bacillus icteroides* has, after all, something to do

with yellow fever. The correct position may possibly be that taken by Guiteras (1909), who is inclined to make Sanarelli's bacillus and other bacteria responsible for the haemorrhagic phenomena in yellow fever; in that case the haemorrhages in yellow fever would have to be regarded as complications and not as mere symptoms.

According to Reed and Carroll (1900) *Bacillus icteroides* belongs to the hog-cholera group, a point of considerable interest, since the *Bacillus suipestifer* has likewise been reduced to a secondary position, whilst it was formerly considered to be the pathogenic microbe of hog-cholera. The virus of hog-cholera is, according to Dorset, Bolton and McBryde (1905), Uhlenhuth (1911) and others, capable of passing through fine porcelain filters, whilst *Bacillus suipestifer* is believed to be the cause of certain complications, and occasionally even the direct cause of death.

The study of Sanarelli's bacillus may consequently be of interest, even when the question of the etiology of yellow fever has been definitely settled.

Organisms, presumably belonging to the Protozoa, have been described as pathogenic agents in yellow fever by various observers, as Klebs (1898), Lacerda (1893), Pothier, Hume, Watson and Couret (1905), Schüller (1906), and Thayer (1907). In my first paper on this subject (1909), I referred to the publication of Pothier, Hume, Watson and Couret, and stated that the bodies described appeared, according to their figures, to be exceptionally well-developed blood platelets. Since then, one of the authors, Dr. Couret, on a visit to Liverpool, admitted that the drawings really were similar to platelets, and that some of the supposed parasites observed might have been so, but, on the other hand, he asserted that they had observed elements which he could identify with those which I have described and which I demonstrated to him, and also that in New Orleans they had been able to diagnose yellow fever by their presence in the blood. It is, therefore, possible that these authors have observed, before me, the yellow fever bodies which I described three years later, but I do not think the impression

was likely to be conveyed, through their description or figures, that they really had to do with such definite elements.

Schüller described both schizonts, in all stages of evolution, and gametes, and, moreover, spirochaetoid forms, the significance of which could not be determined. All these forms were observed in two blood smears, which had been sent to Schüller from New Orleans, or rather in a single smear, since one only was used for staining. The so-called parasites were readily seen in the unstained specimen also, though it is well known that no good results can be expected from the examination of dry films without staining. According to Schüller's description, the elements observed by him were somewhat numerous, large and easily stained; they could, therefore, hardly have been overlooked by the numerous observers working, at that time, on yellow fever in New Orleans. All this considered, much importance cannot be attached to Schüller's discovery.

The intracellular and free corpuscles found by Thayer in the organs, from a case of yellow fever, and described under the name of *Amoeba febris flavae* may, or may not, have been identical with those observed by me. Their large numbers and often considerable size are not in favour of such a supposition.

Stimson (1907) described a spirochaete which he had observed in sections of the kidney from a case of yellow fever. The technique employed was silver impregnation according to Levaditi. With this technique I have not been able to demonstrate any spirochaetoid or other elements which could be taken for protozoal organisms. The *Myxococcidium stegomyiae* described by Parker, Beyer and Pothier (1903) was afterwards found to have no etiological importance.

No definite result had been obtained in spite of considerable efforts, but, according to all probability, the pathogenic organism of yellow fever, an essentially insect-borne disease, would have to be found amongst the Protozoa. This view was also accepted by Finlay (1903) who had, at an earlier date (in a paper written in 1891, but not published until 1903), worked out a hypothesis to explain how the supposed

yellow fever bacterium could be transmitted by means of mosquitos.

This was the position in 1908, when I began my studies on yellow fever, and the microscopic examination of the blood, therefore, quite naturally, formed the first and principal part of my researches. Bacteriological examinations were carried out in a few cases only, and no results worth recording were obtained.

Already, in the first few cases which I examined, I had the good fortune to find a few elements which appeared to be of a protozoal nature and which belonged, as it resulted afterwards, to the largest forms observed. Continuing my observations, I gradually learned to detect much smaller bodies also, which previously I had entirely overlooked. In my first report (1909) mention was made of positive findings in more than fifty cases; later on (1911, ¹), I could add twenty-five positive results in twenty-seven cases, examined under satisfactory conditions, besides six negative cases, in which the technique was deficient. Since then I have examined specimens from thirteen cases, with positive results in twelve. This gives us thirty-seven positive results in forty cases diagnosed, clinically or post-mortem, as yellow fever, and tabulated. If we include the earlier cases, the six cases with deficient technique and one case previously reported (1911, ¹), in which the diagnosis of yellow fever was not accepted officially, we have about ninety cases with positive findings out of a total slightly above one hundred.

This is a very considerable amount of material, especially when it is considered that in many cases a careful search of several slides has been necessary in order to find a single or a few parasites. So far no other observers have published any confirmatory results, an unfortunate fact, the importance of which is, however, somewhat diminished, when it is considered that few investigators seem, at the present time, to be engaged in the microscopic study of yellow fever. According, however, to information from Dr. Hernandez in Mérida, the same bodies have been found by him in several cases of yellow fever.

The essential question is whether sufficient evidence exists to prove the parasitic nature of the bodies described. An examination of the accompanying plate will, I believe, make it evident that we have not to deal with either artefacts, blood-platelets or basophile granules. The only elements which really need be considered are nuclear fragments, both because such have, no doubt, repeatedly been taken for parasites, and especially because V. Schilling (1911,²) has recently suggested that this has been the case in my observations. The material which forms the basis for Schilling's suggestion, has not yet been published in detail; thus a direct answer cannot be given; but I may expressly state that I have examined, in the same place where my yellow fever studies were made, numerous cases of anaemia without finding any elements identical with the yellow fever bodies. Nuclear fragments were often seen, besides typical erythroblasts, but they differed considerably from the yellow fever bodies, being more sharply limited and staining more deeply. A continuous transition was seen from minute granules to well-defined nuclei, and none of these elements did ever show the staining reactions of protoplasm. In the case of the yellow fever bodies, on the other hand, a characteristic separation into chromatin and cytoplasm is a prominent feature. In the larger forms the cytoplasm is generally well defined, and, whilst it may be very scanty or entirely absent in the smallest forms, there are, often in the same blood-smear, transitional forms from such small and pale chromatin-granules to the larger forms with well-developed cytoplasm. But in such smears no transition is seen to larger, deeply-staining nuclear granules, or to typical nuclei of erythroblasts, both of which are, as a rule, entirely absent. My experience is, therefore, decidedly against the assumption that the yellow fever bodies represent degeneration products. As a whole, it has always been a matter of surprise, to myself as well as to other observers, that very few degenerative or regenerative phenomena are observed in the blood in yellow fever. A comparison of the figures so far published by V. Schilling

(1911,¹), with those in this paper, shows considerable differences; I do not deny, however, that occasionally diagnostic difficulties might arise, but this is only what would be expected in the case of such small parasites.

With regard to certain other objections which have been made to the significance of my findings, a few words may be said in addition to what has been said already in my previous papers (1909, 1911,^{1 & 2}). It is true that the supposed parasite has been found in the blood, and in the organs, in small numbers only. This does not exclude that still smaller elements, which have so far not been observed, may be present and more numerous. Otto and Neumann (1905), examining yellow fever blood with the ultra-microscope, found small motile elements in the serum, but which apparently were not of a specific nature; they came to the conclusion that the apparatus was so far of little value for investigations of that kind, and in particular found it useless for the detection of intracorpuseular parasites, as the reflection from the surface of the erythrocytes was exceedingly strong. The presence of extremely small, free, forms of the parasite remains, consequently, an open question. They may be presumed to exist, because yellow fever serum remains infective after having been filtered, as mentioned previously, but their existence does not exclude, of course, that there may be larger forms of the parasite also. This answers another objection which has been raised, that one would not expect to find a visible parasite in yellow fever. It is interesting to note that other investigators, and precisely those who have experimented with filtered serum, hold exactly the same view of this question as I have expressed on various occasions, without having seen their paper. Rosenau, Parker, Francis and Beyer (1904) say (on page 100):—

‘Because the virus of an infectious disease passes a Berkefeld or Pasteur-Chamberland B filter it does not necessarily follow that the parasite which passed the filter is “ultramicroscopic,” or that it may not have elsewhere another phase in its life cycle of large size.’

Returning to the number of parasites present, it must also

be remembered that we know very little about the relationship between the numbers of microbes in a given infection and the intensity of the intoxication produced. It is easy to illustrate this by examples. In an intoxication of the severest possible nature, as tetanus, the number of specific bacilli may be so small that it is difficult to demonstrate their presence. And in protozoal diseases, due to blood-inhabiting parasites, as malaria and various forms of trypanosomiasis, it is a well-known fact that it may be difficult or impossible to find any parasites in the peripheral blood, even during the acute stages.

The lack of correspondence between the period of three days during which the blood in yellow fever is regarded as infective, and the time during which the parasite has been found in the blood (at least eight days in severe cases) may be explained in one of two different ways. Either the forms encountered at the later stages of the disease are not capable of transmitting the infection, or the dogma of the three days is incorrect. This latter part of the question has been discussed in the section on transmission.

To the description of the parasite given in my previous papers, I have but little to add. In Plate I, I have endeavoured to arrange the figures in such a way as to convey a general impression of what appears to be at least a part of its evolution, although all these stages have never been observed in one and the same case. During the earlier days of the disease there appear extremely small chromatin-granules without or with only a faint trace of cytoplasm (figs. 1-2), and in very small numbers somewhat larger forms with more abundant cytoplasm (figs. 3-6). Still larger forms (figs. 11-14, 16 and 19) are found during the later days, and in sections of various organs from a number of autopsies similar parasites have been observed (Figs. 7-10, 15, 34-35), some of them apparently in more advanced stages of evolution. All these forms would seem to form a series of schizonts, gradually increasing in size. It might then be expected that the schizogony would take place in the internal organs and, in fact, the presence of bodies as those depicted in figs. 32,

33 and 45, and also in fig. 43 of my previous paper (1911, ¹) apparently supports this hypothesis, their appearance strongly resembling different stages of division. This apparent formation of merozoites has hitherto been observed in the kidney only, but it may be noted that the bone-marrow has, in most cases, not been examined. The double chromatin granules, on the other hand, which are frequently seen (figs. 20-22), seem to indicate that the process of schizogony may begin in the peripheral blood. In the second period of the disease other elements (figs. 25-31) make their appearance besides those already mentioned; their different structure and staining reactions make it probable that they are not of the same nature, but possibly microgametes, considering their considerable chromatin-contents. A small number of large elements (figs. 36 and 37) are also seen which, on account of their abundant protoplasm, give the impression of macrogametes. Most of the parasites belonging to the latter group are extra-corpuseular, but it does not seem unlikely that they may have been, at an earlier stage of their development, enclosed in the erythrocytes. The form depicted in fig. 36, which is unique, might represent a transitional stage, the parasite just being in the process of escaping from the erythrocyte. It has been a matter of considerable discussion, whether this body is really extra-corpuseular, as it was reproduced in my earlier paper (1911, ¹), or enclosed in an almost decolourized portion of the erythrocyte. The latter view being held by several eminent observers, who have examined my preparations, I have resolved to reproduce the same parasite once more, showing the aspect which it has when examined with considerably subdued light. A similar appearance can also be discerned in a colour-photograph which M. Jeantet, of the Institut Pasteur, kindly made for me two years ago; in a lithograph it is, of course, a matter of great difficulty to make the extremely delicate outlines visible without exaggerating them. In fig. 37 the parasite might just have completed the process of escaping from the blood corpuscle; others are quite free. Two nuclei are observed in fig. 36, and the aspect of the nucleus to the right

seems to indicate that a further division is already in progress. Similar phenomena have never been observed in free forms in the peripheral blood, but bodies with two, three, or even more nuclei have been seen repeatedly in sections. They would correspond to the divisions which are often seen in protozoa previous to the formation of gametes.

The nature of such small free bodies as the one shown in Fig. 38 is entirely doubtful.

Inclusion of parasites in endothelial cells (figs. 39 and 40) is not infrequently seen; whether it is of the nature of a phagocytosis or simply an invasion of the cells cannot be determined.

The absence of a definite chromatin staining in some cases (figs. 17 and 18) is probably a phenomenon of degeneration.

My observations have furnished no clue to the problem of which forms are capable of transmitting the infection when taken up by the mosquito. One would naturally suspect the sexual forms, but the only elements which are at all similar to gametes do not appear in the blood until at a period when the blood is not believed to be infective.* This question must be left entirely to future investigations.

The transmission, on the other hand, by means of serum-injections would probably depend on the presence of free merozoites. In their free state it would be exceedingly difficult to observe such minute elements, but it does not seem unlikely that elements as those shown in fig. 32, which have a transverse diameter of about $0.2\ \mu$, and which, moreover, would probably be plastic, might pass through the pores of a porcelain filter.

The foregoing considerations are to a large extent hypothetical; this could not have been otherwise, as the observations do not form an uninterrupted chain. These considerations may, on further investigation, prove wrong, but I believe that there is strong evidence in support of the fundamental point that yellow fever is caused by a protozoal

* It may be of interest to mention that Finlay (1903) considers it likely that the yellow fever parasite passes its sexual cycle in the human host, but the asexual cycle in the *Stegomyia*.

parasite, the principal forms of which have now been described.

The relationship of this parasite to other blood-inhabiting Protozoa cannot be determined before a more detailed knowledge has been obtained of its different phases, especially of those in the mosquito, which may be presumed to be its definite host, i.e., to harbour the parasite during the time of its sexual evolution.

The parasite differs in many respects from the genus *Plasmodium*, particularly in the lack of pigment; at least, I have never observed with certainty any pigmented forms, though one case has seemed doubtful with regard to this point.

A closer resemblance exists, morphologically, to various forms of *Babesiidae*, especially to *Theileria parva*. Several points in the recent paper by Gonder (1911) on the evolution of *Theileria parva* are demonstrative of this. Thus, of his figures of intra-corpuseular parasites many are very similar to my own. Moreover, the intra-cellular division forms (Koch's granules) are not unlike groups of parasites which I have previously described, and I may add that lately I have observed in well-stained sections intra- and extra-cellular granules, figures of which I, however, do not reproduce at present, not feeling convinced of their parasitic nature. It will be remembered that the parasitic nature of Koch's granules and even their specific importance in East Coast fever has been contested by Mayer (1910) and others, while it is upheld by Gonder (*op. cit.*) and Meyer (1911). Another point is that the comparatively large free bodies, with two or several nuclei which I have mentioned in the description, have considerable resemblance to the 'free gamogonous forms' of Gonder. Biologically, it is interesting that both in East Coast fever and in yellow fever the parasites seem to exert but slight deleterious influence on the erythrocytes; also that the life of the parasite in its mammalian host is said to be of short duration in East Coast fever, which would correspond to what is commonly accepted for yellow fever.

Some forms of the yellow fever parasite have a certain

resemblance to Theiler's (1910, ^{1 & 2}) *Anaplasma marginale*, and to the bodies found by Bruce and collaborators (1910) in cattle in Uganda.

These resemblances make it probable that the yellow fever parasite belongs to the family of *Babesiidae*, but it would not be justifiable to include it in any of the established genera. I, therefore propose the name of

Paraplasma flavigenum, g. n., sp. n.,

thus giving expression in the specific name to its alleged importance as the cause of yellow fever.

Type specimens have been deposited in the collection of the Liverpool School of Tropical Medicine.

Blood-parasites, which might perhaps be related to those of yellow fever, have been described as the cause of various diseases in man, namely, in dengue by Graham (1902) and Eberle (1904), in spotted fever by Wilson and Chowning (1903), confirmed by Anderson (1903), and in exanthematic typhus by Gotschlich (1903) and Krompecher, Goldzieher and Augyán (1909). These observations have not been confirmed, but, on the other hand, cannot be regarded as disproved. On clinical and epidemiological grounds a relationship has been assumed, by various authors, to exist between yellow fever, dengue and papataci fever, and it seems not unlikely that these three diseases may be caused by parasites belonging to one and the same group. On the other hand, several reasons, which I have formerly alluded to, indicate that yellow fever may not be exactly the same disease in all places where it occurs; this would be explained if various forms of *Paraplasma* exist, just as various types of malaria are caused by different species of *Plasmodium*. Finally, I have observed a case, to be reported elsewhere, which on both clinical and parasitological observation proved similar to, but not identical with, yellow fever. Thus, there is some reason to suspect that we shall have to consider, in future, different varieties of paraplasmosis, as well as we speak of different babesioses and trypanoses.

It is a question of the utmost importance whether the demonstration of *Paraplasma flavigenum* can be used as a

means of diagnosis. At the present we know of no pathognomonic sign in yellow fever, and in atypical cases it may be impossible to prove the diagnosis. The high percentage of positive findings in my cases makes it probable that the parasite may, under favourable conditions, prove of constant occurrence. In my earlier cases it may easily have been overlooked, and in my last series the blood-smears were sent to me from Mérida, and stained several weeks afterwards, in Liverpool, which, of course, makes it impossible to obtain uniformly good staining results. Before arriving at a conclusion as to the diagnostic importance, several difficulties have to be considered:—

I. There is a possibility, as stated, that some other diseases may be caused by similar parasites. Should this be found to be the case, the differential diagnosis of these parasites must be worked out; it is possible that no distinctive characters might be found, but then a positive finding would, at any rate, have the advantage of limiting the clinical consideration to a comparatively small group of diseases. Another question is, whether we shall have to count with the existence of microbe-carriers, as in malaria and many forms of babesiosis as well as in numerous bacterial diseases. According to Marchoux and Simond (1906) and others, natives and immunized foreigners may become infected repeatedly with yellow fever microbes and present very slight or no symptoms at all; they would not become microbe-carriers in the ordinary sense of the word, but, practically, the result would be the same, since in both cases we might find parasites in the blood of apparently healthy individuals. As mentioned before (1911, ¹), I believe that I have observed parasites in two natives without symptoms of yellow fever, but the occurrence would seem quite exceptional, as these were the only cases amongst many hundreds of blood examinations. This circumstance might, of course, at first make the diagnosis difficult, but, finally, it would be a great advantage to be able to find out the microbe-carriers, the most dangerous individuals from an epidemiological point of view.

II. The number of parasites may be so small that they may easily escape detection, when the examiner has not unlimited time at his disposal, which is seldom the case in routine work. Unfortunately, the larger forms, which are easily seen, are also the most scarce, seldom more than one or two in a slide-preparation, and in some cases a single parasite has been found after a careful search of several smears; on the other hand, the small forms, which may be more numerous, occasionally 30 to 50 in an ordinary smear, are likely to escape observation on account of their minute dimensions.

III. In order to find even these small elements the microscopic outfit must be the best possible. I have demonstrated many parasites with an ordinary 1/12 oil-immersion lens, in specimens previously marked, but for diagnostic purposes such a system must be regarded as quite unsatisfactory. Most of my work has been done with a Zeiss 3 mm. apochromatic oil-immersion lens and a No. 12 compensation eye-piece, a system which does not give a very high magnification ($\times 1000$ with ordinary tube-length), but very sharp images. When studying details, a No. 18 eye-piece may be substituted, or a 2 mm. or 1.5 mm. lens may be used, the latter giving, with a No. 18 eye-piece, a magnification of 3000, but its resolving power is not so high, and to search a whole blood-smear with this system would be a very slow process.

A considerable inconvenience is the high cost of the apochromatic lenses, but this consideration ought to be subordinate in comparison with the great importance of the diagnosis.

IV. The blood-smears must be well prepared, and the stain both intense and without precipitates. The drop of blood must be small, and when spreading it out on the slide, it is advantageous to move the border of the second slide, or the glass rod, which is used for spreading, away *from* the drop, instead of towards it, as is most frequently done. Dried smears should be fixed for about an hour, preferably in absolute methyl-alcohol. So-called wet films would be useful

for the further study of the parasite, but require more time and attention to details; they are, therefore, less convenient for routine diagnostic work.

Dried smears should be stained, whenever possible, for twenty-four hours in a diluted Giemsa's solution, 1 : 20 (one drop per c.c. of distilled water) or, better still, for one to two hours in this dilution and subsequently twenty hours in a dilution of 1 to 30 or 40. If necessary, a cautious differentiation with acetone may be used. When a rapid diagnosis is essential, the staining may take place in a somewhat stronger solution (15 drops to 10 c.c.) for two hours. Also the 'quick methods,' combining fixation and staining of Leishman, Wright and Giemsa, may be employed, but the results cannot be expected to be always satisfactory.

Wet films and sections are similarly stained; I have recently (1911,³) described what I considered the best technique for this purpose, but have since substituted Pappenheim's Panchrom solution for Giemsa's solution. Thus, the following technique may now be recommended:—

1. *Thin* pieces of tissue, as fresh as possible, into a mixture of watery saturated mercury bichloride solution 2 pts., alc. abs. 1 pt., for twenty-four hours.
2. Twenty-four hours in each of the following liquids: 60 % alc., 70 % alc. with tincture of iodine, 3-5 drops to 10 c.c., 90 % alc., abs. alc.; through xylol to paraffin; embedding.
3. Sections 5 μ in thickness, or less; through xylol and alcohols of diminishing strength to tap-water.
4. Diluted Lugol's solution (2 c.c. to 100 c.c. of water), five to ten minutes.
5. Tap-water, five minutes.
6. 0.5% solution of sodium thiosulphate (sodium hyposulphite), five to ten minutes.
7. Tap-water, five minutes or more.
8. Diluted Panchrom solution,* 1:20 in distilled water, which has been neutralized, if necessary, with potassium carbonate, using a haematoxylin indicator, as recommended

*Pappenheim's Panchrom für Schnittfärbung from Dr. G. Grüber, Leipzig.

by Giemsa (to 10 c.c. of water add two drops of haematoxylin in 96 % alc.; if it turns a faint blue in from one to five minutes, it can be used without addition, if not, add a few drops of a 1 % potassium carbonate solution till that point is reached), one to two hours.

9. The same, 1 : 40.
10. Wash in tap-water.
11. Differentiate in pure acetone (control under microscope).
12. Acetone 70, xylol 30, five minutes.
13. Acetone 30, xylol 70, five minutes.
14. Xylol, five minutes.
15. Fresh xylol.
16. Damarlack.

The differentiation should be carried so far that the desired result is almost completely obtained; practically no differentiation should take place in the acetone-xylol mixtures. The results can often be as perfect as those obtained in good smears, but are not quite as constant.

In the preparation of wet films the fixing and hardening procedures can be somewhat abbreviated, but no harm is done by following exactly the same technique.

The iron-haematein method has given fairly good results, as shown in figs. 41-45, but can never be of the same use for diagnostic purposes as the Romanowsky stains, because it does not give the same striking differentiation of chromatin and protoplasm.

There will be, undoubtedly, great difficulties in establishing the microscopic diagnosis in cases of yellow fever, but this only means that a continued careful study of the matter is necessary. So far, only in a few cases has the microscopical diagnosis been made before the clinical diagnosis was established.

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EXPLANATION OF PLATE

The figures have been drawn with Abbe's camera lucida, using Zeiss's apochr. imm. 3 mm. and compens. oc. 12. The magnification varies from 1,300 to 1,400 lin., according to the height of the drawing table.

Figs. 7-10, 15, 32-35 and 39-45 are from sections (stomach : 7, 8, 10, 41-45, liver : 39 and 40, kidney : 32 and 33, spleen : 34 and 35, thyroid body : 9 and 15), all others from the peripheral blood.

Fig. 1—Naked chromatin granule.

Figs. 2-5—Chromatin granule with linear protoplasma-body ; in 5, slightly curved.

Figs. 6-16—Parasites gradually increasing in size, especially their protoplasm.

Figs. 17 and 18—Protoplasma-bodies without chromatin. (Degeneration forms ?)

Fig. 19—Beginning division of chromatin.

Figs. 20-28—Chromatin double ; in 22, apparently beginning division of protoplasm.

Fig. 23—Double form.

Fig. 24—Chromatin separated from protoplasm. (Degeneration ?)

Figs. 25-28—Chromatin bodies without evidence of protoplasm.

Figs. 29-31—Similar bodies dividing.

Figs. 32 and 33—Multiple division ?

Figs. 34 and 35—Large forms with abundant protoplasm, in spleen.

Fig. 36—Large form, apparently escaping from erythrocyte.

Fig. 37—Large free form.

Fig. 38—Small free form.

Figs. 39 and 40—Endothelial cells with parasites.

Figs. 1-40—Are all stained with Giemsa's or panchrom-solution.

Figs. 41-45—Parasites as seen with the iron-haematein stain ; in 43 and 44 double infection of erythrocytes, in 45 either multiple infection or division.



